CLAIMS

WHAT IS CLAIMED IS:

1. An *ex vivo* method for selectively activating and expanding γδ T cells in an isolated mixed cell population, the method comprising contacting the isolated mixed cell population with one or more soluble multivalent agents that selectively activate and expand γδ T cells by binding to at least one epitope of a γδ TCR.

2. An *ex vivo* method for selectively activating and expanding one or more γδ T cell subtypes in an isolated mixed cell population, the method comprising contacting the isolated mixed cell population with one or more soluble multivalent agents that selectively activate and expand δ1 T cells, δ2 T cells, or δ3 T cells, or a combination thereof, preferably wherein the one or more agents that selectively activate and expand δ1 T cells bind to an activating epitope specific of a δ1 TCR; the one or more agents that selectively activate and expand δ2 T cells bind to an activating epitope specific of a δ2 TCR; and the one or more agents that selectively activate and expand δ3 T cells bind to an activating epitope specific of a δ3 TCR, thereby activating and expanding the desired γδ T cell subtype in the mixed cell population.

3. An *in vitro* and/or *ex vivo* method for producing an enriched γδ T-cell population comprising directly contacting an isolated mixed cell population comprising γδ T-cells, or a purified fraction thereof, with one or more soluble multivalent agents; preferably wherein the soluble multivalent agent(s) activate and expand γδ T cells by binding to at least one epitope of a γδ TCR.

4. An *in vitro* and/or *ex vivo* method for producing enriched γδ T-cell sub-populations from isolated mixed cell populations, comprising directly contacting an isolated mixed cell population with one or more soluble multivalent agents that i) selectively expand δ1 T-cells by binding to an epitope specific of a δ1 TCR, ii) that selectively expand δ2 T cells by binding to an epitope specific of a δ2 TCR, and iii) that selectively expand δ3 T cells by binding to an epitope specific of a δ3 TCR, to provide an enriched γδ T cell sub-population.

5. The method according to any preceding claim, wherein the soluble multivalent agent comprises at least two antigen-binding sites that specifically bind the same antigen, or wherein the

multivalent agent comprises at least two antigen-binding sites that specifically bind the same epitope of the same antigen.

6. The method according to claim 5, wherein the soluble multivalent agent is, or is at least, bivalent, trivalent, or tetravalent, and optionally monospecific.

7. The method according to any preceding claim, wherein the soluble multivalent agent is tetravalent.

8. The method according to any preceding claim, wherein the soluble multivalent agent is monospecific.

9. The method according to claim 5, wherein the antigen-binding sites bind to different epitopes on the constant or variable regions of δ TCR and/or γ TCR.

10. The method according to claim 5, wherein the antigen-binding sites comprise the CDRs from γδ TCR pan MAbs, preferably wherein the antigen-binding sites specifically bind domains shared by different γ and δ TCRs on either the γ or δ chain or both, including δ1, δ2, and δ3 T cell populations.

11. The method according to claim 5, wherein the soluble multivalent agent selectively expands δ1 T-cells and comprises at least two, or greater than two, antigen-binding sites that specifically bind to an epitope comprising a δ1 variable region.

12. The method according to claim 11, wherein the soluble multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind a δ1 TCR Bin 1 δ1 epitope, Bin 1b δ1 epitope, Bin 2 δ1 epitope, Bin 2b δ1 epitope, Bin 2c δ1 epitope, Bin 3 δ1 epitope, Bin 4 δ1 epitope, Bin 5 δ1 epitope, Bin 6 δ1 epitope, Bin 7 δ1 epitope, Bin 8 δ1 epitope, or a Bin 9 δ1 epitope of a human δ1 TCR.

13. The method according to claim 11, wherein the soluble multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind to the same, or essentially the same, epitope as, or competes with, an antibody selected from the group consisting of δ1-05, δ1-08, δ1-18, δ1-22, δ1-23, δ1-26, δ1-35, δ1-37, δ1-39, δ1-113, δ1-143, δ1-149, δ1-155, δ1-182, δ1-183,

δ1-191, δ1-192, δ1-195, δ1-197, δ1-199, δ1-201, δ1-203, δ1-239, δ1-253, δ1-257, δ1-278, δ1-282, and δ1-285.

14. The method according to claim 11, wherein the soluble multivalent agent comprises the CDRs of an antibody selected from the group consisting of δ1-05, δ1-08, δ1-18, δ1-22, δ1-23, δ1-26, δ1-35, δ1-37, δ1-39, δ1-113, δ1-143, δ1-149, δ1-155, δ1-182, δ1-183, δ1-191, δ1-192, δ1-195, δ1-197, δ1-199, δ1-201, δ1-203, δ1-239, δ1-253, δ1-257, δ1-278, δ1-282, and δ1-285.

15. The method of claim 11, wherein the soluble multivalent agent comprises the CDRs of antibody δ1-35 or δ1-08, or binds the same epitope as antibody δ1-08 or δ1-35.

16. The method according to claim 11, wherein the soluble multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind the same epitope as an antibody selected from TS-1 and TS8.2.

17. The method according to claim 16, wherein the soluble multivalent agent comprises the CDRs of TS-1 or TS8.2 and/or is a humanized TS-1 or TS8.2.

18. The method according to claim 11, wherein the soluble multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind to an epitope comprising residues Arg71, Asp72 and Lys120 of the δ1 variable region.

19. The method according to claim 5, wherein the soluble multivalent agent selectively expands δ1 T-cells and δ3 T cells.

20. The method according to claim 5, wherein the soluble multivalent agent selectively expands δ1 T cells selectively expands δ1, δ3, δ4, and δ5 γδ T cells.

21. The method according to claim 5, wherein the soluble multivalent agent selectively expands δ2 T-cells and comprises at least two, or greater than two, antigen-binding sites that specifically bind to an epitope comprising a δ2 variable region.

22. The method according to claim 21, wherein the soluble multivalent agent comprises at least two, or greater than two, antigen-binding sites that have reduced binding to a mutant δ1 TCR polypeptide comprising a mutation at K120 of delta J1 and delta J2.

23. The method according to claim 21, wherein the soluble multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind a δ2 TCR Bin 1 δ2 epitope, Bin 2 δ2 epitope, Bin 3 δ2 epitope, or Bin 4 δ2 epitope of a human δ2 TCR.

24. The method according to claim 21, wherein the soluble multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind to the same, or essentially the same, epitope as, or competes with, an antibody selected from the group consisting of δ2-14, δ2-17, δ2-22, δ2-30, δ2-31, δ2-32, δ2-33, δ2-35, δ2-36, and δ2-37.

25. The method according to claim 24, wherein the soluble multivalent agent comprises the CDRs of an antibody selected from the group consisting of δ2-14, δ2-17, δ2-22, δ2-30, δ2-31, δ2-32, δ2-33, δ2-35, δ2-36, and δ2-37.

26. The method of claim 21, wherein the soluble multivalent agent comprises the CDRs of antibody δ2-37 or binds the same epitope as antibody δ2-37.

27. The method according to claim 21, wherein the soluble multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind to the same epitope as an antibody selected from 15D and B6.

28. The method according to claim 26, wherein the soluble multivalent agent comprises the CDRs of antibody 15D or B6 and/or is a humanized 15D and B6 antibody.

29. The method according to claim 21, wherein the soluble multivalent agent comprises at least two, or greater than two, antigen-binding sites that have reduced binding to a mutant δ2 TCR polypeptide comprising a mutation at G35 of the δ2 variable region.

30. The method according to claim 5, wherein the soluble multivalent agent selectively expands δ3 T-cells and comprises at least two, or greater than two, antigen-binding sites that specifically bind to an epitope comprising a δ3 variable region.

31. The method according to claim 30, wherein the soluble multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind to the same, or essentially the same, epitope as, or competes with, an antibody selected from the group consisting of δ3-08, δ3-20, δ3-23, δ3-31, δ3-42, δ3-47 and δ3-58.

32. The method according to claim 31, wherein the soluble multivalent agent comprises the CDRs of an antibody selected from the group consisting of δ3-08, δ3-20, δ3-23, δ3-31, δ3-42, δ3-47 and δ3-58.

33. The method according to any preceding claim, wherein the method comprises simultaneously or sequentially culturing the γδ T-cell population with a cytokine, preferably wherein the cytokine is a common gamma chain cytokine.

34. The method according to claim 33, wherein the cytokine is selected from the group consisting of IL-2, IL-7, IL-9, IL-12, IL-15, IL-18, IL-19, IL-21, IL 23, and IL-33, preferably wherein the cytokine is IL-2, IL-15, IL-12, or IL-21.

35. The method according to any preceding claim, wherein the γδ T-cell population is engineered before and/or after activation and/or expansion.

36. The method according to claim 35, wherein the γδ T-cell population is engineered to stably express one or more tumor recognition moieties encoded by expression cassettes, and/or wherein the γδ T-cell population is engineered to stably express a transgene that encodes at least one secreted cytokine.

37. The method according to claim 36, wherein the at least one secreted cytokine is a common gamma chain cytokine selected from the group consisting of IL-2, IL-15 and IL-4, preferably wherein the cytokine is IL-2, IL-15 or IL-4.

38. The method according to any preceding claim, wherein the method further comprises administering the expanded / enriched engineered and/or non-engineered γδ T cell populations to a patient in need thereof, preferably wherein the administered population of γδ T cells comprises at least 60% γδ T cells.

39. The method according to claim 37, wherein the administered population of engineered and/or non-engineered γδ T cells is a population of cells that are autologous to the subject.

40. The method according to claim 37, wherein the administered population of engineered and/or non-engineered γδ T cells is a population of cells that are allogeneic to the subject.

41. The method according to any preceding claim, further comprising performing a depletion step for αβ T cells after activation and expansion of the γδ T-cell population, and before administration to the subject.

42. A soluble composition comprising one or more multivalent agent(s) for selectively activating and expanding γδ T cells, a subtype thereof, or combinations thereof, wherein said multivalent agent(s) comprises at least two antigen-binding sites that specifically bind the same epitope of a γδ TCR; preferably wherein the multivalent agent is, or is at least, bivalent, trivalent, tetravalent, or pentavalent, and optionally monospecific.

43. The soluble composition according to claim 42, wherein the multivalent agent is tetravalent.

44. The soluble composition according to 42 or 43, wherein the multivalent agent is monospecific.

45. The soluble composition according to claim 42, wherein the antigen-binding sites comprise the CDRs from γδ TCR pan MAbs, preferably wherein the multivalent agent comprises the amino acid sequence set forth in Fig. x or y.

46. The soluble composition according to claim 42, wherein the multivalent agent selectively expands δ1 T-cells and comprises at least two, or greater than two, antigen-binding sites that specifically bind to an epitope comprising a δ1 variable region.

47. The soluble composition according to claim 46, wherein the multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind a δ1 TCR Bin 1 δ1 epitope, Bin 1b δ1 epitope, Bin 2 δ1 epitope, Bin 2b δ1 epitope, Bin 2c δ1 epitope, Bin 3 δ1 epitope, Bin 4 δ1

epitope, Bin 5 δ1 epitope, Bin 6 δ1 epitope, Bin 7 δ1 epitope, Bin 8 δ1 epitope, or a Bin 9 δ1 epitope of a human δ1 TCR.

48. The soluble composition according to claim 46, wherein the multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind to the same, or essentially the same, epitope as, or competes with, an antibody selected from the group consisting of δ1-05, δ1-08, δ1-18, δ1-22, δ1-23, δ1-26, δ1-35, δ1-37, δ1-39, δ1-113, δ1-143, δ1-149, δ1-155, δ1-182, δ1-183, δ1-191, δ1-192, δ1-195, δ1-197, δ1-199, δ1-201, δ1-203, δ1-239, δ1-253, δ1-257, δ1-278, δ1-282, and δ1-285.

49. The soluble composition according to claim 46, wherein the multivalent agent comprises the CDRs of an antibody selected from the group consisting of δ1-05, δ1-08, δ1-18, δ1-22, δ1-23, δ1-26, δ1-35, δ1-37, δ1-39, δ1-113, δ1-143, δ1-149, δ1-155, δ1-182, δ1-183, δ1-191, δ1-192, δ1-195, δ1-197, δ1-199, δ1-201, δ1-203, δ1-239, δ1-253, δ1-257, δ1-278, δ1-282, and δ1-285.

50. The soluble composition of claim 46, wherein the multivalent agent comprises the CDRs of antibody δ1-35 or δ1-08, or binds the same epitope as antibody δ1-08 or δ1-35, preferably wherein the multivalent agent comprises the amino acid sequence set forth in Fig. XX.

51. The soluble composition according to claim 46, wherein the multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind the same epitope as an antibody selected from TS-1 and TS8.2.

52. The soluble composition according to claim 51, wherein the soluble multivalent agent comprises the CDRs of TS-1 or TS8.2 and/or is a humanized TS-1 or TS8.2.

53. The soluble composition according to claim 46, wherein the multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind to an epitope comprising residues Arg71, Asp72 and Lys120 of the δ1 variable region.

54. The soluble composition according to claim 42, wherein the multivalent agent selectively expands δ1 T-cells and δ3 T cells.

55. The soluble composition according to claim 42, wherein the multivalent agent selectively expands δ1 T cells selectively expands δ1, δ3, δ4, and δ5 γδ T cells.

56. The soluble composition according to claim 42, wherein the multivalent agent selectively expands δ2 T-cells and comprises at least two, or greater than two, antigen-binding sites that specifically bind to an epitope comprising a δ2 variable region.

57. The soluble composition according to claim 56, wherein the multivalent agent comprises at least two, or greater than two, antigen-binding sites that have reduced binding to a mutant δ1 TCR polypeptide comprising a mutation at K120 of delta J1 and delta J2.

58. The soluble composition according to claim 56, wherein the multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind a δ2 TCR Bin 1 δ2 epitope, Bin 2 δ2 epitope, Bin 3 δ2 epitope, or Bin 4 δ2 epitope of a human δ2 TCR.

59. The soluble composition according to claim 56, wherein the multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind to the same, or essentially the same, epitope as, or competes with, an antibody selected from the group consisting of δ2-14, δ2-17, δ2-22, δ2-30, δ2-31, δ2-32, δ2-33, δ2-35, δ2-36, and δ2-37.

60. The soluble composition according to claim 59, wherein the multivalent agent comprises the CDRs of an antibody selected from the group consisting of δ2-14, δ2-17, δ2-22, δ2-30, δ2-31, δ2-32, δ2-33, δ2-35, δ2-36, and δ2-37.

61. The soluble composition of claim 56, wherein the multivalent agent comprises the CDRs of antibody δ2-37 or binds the same epitope as antibody δ2-37.

62. The soluble composition according to claim 56, wherein the multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind to the same epitope as an antibody selected from 15D and B6.

63. The soluble composition according to claim 62, wherein the multivalent agent comprises the CDRs of antibody 15D or B6 and/or is a humanized 15D and B6 antibody.

64. The soluble composition according to claim 56, wherein the multivalent agent comprises at least two, or greater than two, antigen-binding sites that have reduced binding to a mutant δ2 TCR polypeptide comprising a mutation at G35 of the δ2 variable region.

65. The soluble composition according to claim 42, wherein the multivalent agent selectively expands δ3 T cells and comprises at least two, or greater than two, antigen-binding sites that specifically bind to an epitope comprising a δ3 variable region.

66. The soluble composition according to claim 65, wherein the multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind to the same, or essentially the same, epitope as, or competes with, an antibody selected from the group consisting of δ3-08, δ3-20, δ3-23, δ3-31, δ3-42, δ3-47 and δ3-58.

67. The soluble composition according to claim 66, wherein the multivalent agent comprises the CDRs of an antibody selected from the group consisting of δ3-08, δ3-20, δ3-23, δ3-31, δ3-42, δ3-47 and δ3-58.

68. Use of a soluble composition according to any one of claims 42-67 in the manufacture of a medicament for treating a cancer, infectious disease, inflammatory disease, or an autoimmune disease in a subject in need thereof, wherein the medicament comprises the resulting expanded γδ T cell population, subtype thereof, or admixtures thereof.